

and J. Munro for permission to study patients under their care, and Mr. J. Barclay and Mr. W. Shade for technical help.

## References

- Allington, M. J. (1967). *British Journal of Haematology*, 13, 550.  
 Black, D. A. K., Rose, G., and Brewer, D. B. (1970). *British Medical Journal*, 3, 421.  
 Brown, J. H., and Mackey, H. K. (1968). *Proceedings of the Society for Experimental Biology and Medicine*, 128, 504.  
 Cash, J. D., and Allen, A. G. E. (1967). *British Journal of Haematology*, 13, 376.  
 Clarkson, A. R., Morton, J. B., and Cash, J. D. (1970). *Lancet*, 2, 1220.  
 Clarkson, A. R., MacDonald, M. K., Petrie, J. J. B., Cash, J. D., and Robson, J. S. (1971). *British Medical Journal*, 3, 447.  
 Das, P. C. (1970). *Journal of Clinical Pathology*, 23, 149.  
 Hawiger, J., Niewiarowski, S., Gurewich, V., and Thomas D. P. (1970). *Journal of Laboratory and Clinical Medicine*, 75, 93.  
 Isaacson, S. (1970). *Scandinavian Journal of Haematology*, 7, 212.  
 Kvarstein, B., and Stormoken, H. (1971). *Biochemical Pharmacology*, 20, 119.  
 Leavelle, D. E., Bowie, E. J. W., Mertens, B. F., McDuffie, F. C., and Owen, C. A. (1971). *Journal of Laboratory and Clinical Medicine*, 77, 993.  
 Lipinski, B., Hawiger, J., and Jeltaszewicz, J. (1967). *Journal of Experimental Medicine*, 126, 979.  
 Marder, V. J., Matchett, M. O., and Sherry, S. (1971). *American Journal of Medicine*, 51, 71.  
 Merskey, C., Kleiner, G. J., and Johnson, A. J. (1966). *Blood*, 28, 1.  
 Michielsen, P., Verberckmoes, R., Desmet, V., and Hemerijckz, W. (1969a). *Journal d'Urologie et de Nephrologie*, 75, 315.  
 Michielsen, P., Verberckmoes, R., and Hemerijckz, W. (1969b). *Proceedings of the IV International Congress of Nephrology, Stockholm*, 3, 92.  
 Northover, B. J. (1971). *British Journal of Pharmacology*, 41, 540.  
 O'Brien, J. R., Finch, W., and Clark, E. (1970). *Journal of Clinical Pathology*, 23, 522.  
 Phelps, P., and McCarty, D. J. (1967). *Journal of Pharmacology and Experimental Therapeutics*, 158, 546.  
 Pollak, V. E., Pirani, C. L., and Kark, R. M. (1961). *Journal of Laboratory and Clinical Medicine*, 57, 495.  
 Smith, J. O., and Willis, A. L. (1971). *Nature New Biology*, 231, 235.  
 Thomas, D. P., Niewiarowski, S., Myers, A. R., Bloch, K. J., and Colman, R. W. (1970). *New England Journal of Medicine*, 283, 663.  
 Vane, J. R. (1971). *Nature New Biology*, 231, 232.  
 Vermynen, J., Dotremont, G., Gaetano, G. de, Donati, M. B., and Michiel- sen, P. (1970). *Revue Européenne d'Etudes Cliniques et Biologiques*, 15, 979.  
 Zucker, M. B., and Peterson, J. (1970). *Journal of Laboratory and Clinical Medicine*, 76, 66.

# Effect of Vegetarianism and Smoking on Vitamin B<sub>12</sub>, Thiocyanate, and Folate Levels in the Blood of Normal Subjects

D. K. DASTUR, E. V. QUADROS, N. H. WADIA, M. M. DESAI, E. P. BHARUCHA

*British Medical Journal*, 1972, 3, 260-263

## Summary

Vitamin B<sub>12</sub>, thiocyanate, and folate levels in the blood were estimated in 69 apparently normal subjects, of whom 26 were non-vegetarian non-smokers, 19 non-vegetarian smokers, 15 vegetarian non-smokers, and nine vegetarian smokers. The serum total (cyanide-extracted) B<sub>12</sub> level (value A) ranged from 105 to 728 pg/ml, with a mean of 292 pg/ml. The highest values were found in non-vegetarian non-smokers and the lowest in vegetarian smokers. There was no significant difference in value A between smokers as a group and non-smokers as a group. On the other hand, in vegetarians value A was very significantly lower than in non-vegetarians regardless of their smoking habits.

It is suggested that A may represent both the protein-bound and free forms of vitamin B<sub>12</sub> in the blood, and B mainly the free B<sub>12</sub>, which may be the physiologically active form. The plasma thiocyanate level varied from 1.0 to 15 μmol/100 ml, being, as expected, much higher in smokers (mean 8.20 μmol/100 ml) than in non-smokers (mean 2.02 μmol/100 ml). There was a rough correlation between falling B<sub>12</sub> levels and rising thiocyanate levels. The serum folate level ranged from 2.75 to 15.75 ng/ml, and was slightly but significantly higher in vegetarians (mean 6.60 ng/ml) than in non-vegetarians (mean 4.79 ng/ml), reflecting the greater content of folate in a vegetarian diet.

## Introduction

It was suggested by Wokes and Picard in 1955 and by Wokes in 1958 that vitamin B<sub>12</sub> contributed to the detoxication of exogenous cyanide, such as that derived from tobacco smoke. Cyanide probably participates in normal metabolic processes (Boxer and Rickards, 1952) and possibly combines with B<sub>12</sub> to form cyanocobalamin. Heaton *et al.* (1958) reported reduced serum B<sub>12</sub> levels in pipe smokers with visual impairment and suggested that this was related to the pathogenesis of tobacco amblyopia.

It was recognized by Spray (1955) and by Boger *et al.* (1955) that unless cyanide was added to the buffer used to extract serum B<sub>12</sub> in the *Lactobacillus leichmannii* assay, the results obtained were spuriously low. Matthews (1961, 1962) clearly showed that this was owing to loss of the vitamin in the protein precipitate. Subsequently, even in their radioisotopic assay, Matthews *et al.* (1967) advocated the use of acetate-cyanide buffer for the extraction of the total cobalamins from the serum.

The estimation of microquantities of cyanide and thiocyanate in the blood was first carried out by Aldridge (1945). Using this method, Wilson and Matthews (1966) found higher plasma thiocyanate values in smokers than in non-smokers, both groups being otherwise normal. They further showed that the proportion of non-CN-extracted serum B<sub>12</sub> was higher in smokers than in non-smokers. They suspected a significant relation between plasma thiocyanate concentration and the level of non-CN-extracted B<sub>12</sub>.

The interrelationships between smoking and serum B<sub>12</sub> levels might be expected to be affected by B<sub>12</sub> intake, which is generally lower in vegetarians than in meat-eaters. Hence the objectives of the investigations reported here were: (1) to observe the serum B<sub>12</sub>, plasma thiocyanate, and serum folate levels in four groups of apparently healthy subjects—non-vegetarian non-smokers, non-vegetarian smokers, vegetarian non-smokers, and vegetarian smokers; and (2) to study the interrelationships between these three compounds, and especially the relation of smoking to the levels of total (CN-extracted) and non-CN-extracted cobalamins.

Grant Medical College and J. J. Group of Hospitals, Bombay-8, India

D. K. DASTUR, M.D., M.R.C. PATH., Head of Neuropathology Unit

E. V. QUADROS, M.Sc., Research Assistant

N. H. WADIA, M.D., F.R.C.P., Head of Department of Neurology

M. M. DESAI, M.B., B.S., Research Fellow

K.E.M. Hospital, Bombay, India

E. P. BHARUCHA, M.D., Head of Department of Neurology

## Subjects and Methods

Of the 69 subjects studied, 39 were drawn from apparently healthy laboratory workers and doctors; the remaining 30 were drawn from patients' relatives, also apparently healthy, who had come to donate blood at the blood bank. None of them had any evidence of anaemia. These 69 subjects fell into the following four groups: (1) 26 non-vegetarian non-smokers, (2) 19 non-vegetarian smokers, (3) 15 vegetarian non-smokers, and (4) 9 vegetarian smokers. All smokers smoked at least 10 cigarettes a day; there were no pipe smokers. All the vegetarians took milk and dairy products and may thus be regarded as lacto-vegetarians. None of these subjects took any vitamin supplements.

Serum B<sub>12</sub> levels were estimated by the isotopic assay described by Matthews *et al.* (1967). Essentially, the procedure consisted in extraction of total cobalamins from the subject's serum with acetate-cyanide buffer at pH 4.5, this being subsequently called "total (CN-extracted) B<sub>12</sub>," or value A. Another part of the serum was treated identically but with acetate buffer alone, this being subsequently called "non-CN-extracted B<sub>12</sub>," or value B. Both these extracts were assayed after the addition of <sup>57</sup>Co-B<sub>12</sub> as the label, normal human serum as the binding protein, and albumin-coated charcoal to separate the bound and free fractions, which were counted in a well-type gamma-ray spectrometer. The free:bound ratio was calculated for both the sample and the standard solutions of B<sub>12</sub> and plotted against concentrations of the standards; the value in the sample was read off from this graph.

Plasma thiocyanate levels were estimated spectrophotometrically by the method of Aldridge (1945) and serum folate levels by a modification of the *L. casei* assay procedure of Baker and Frank (1968).

## Results

SERUM VITAMIN B<sub>12</sub>

As shown in Table I there was a stepwise downward gradation of mean levels of total (CN-extracted) serum B<sub>12</sub> (value A) from the non-vegetarian non-smokers to the non-vegetarian smokers, vegetarian non-smokers, and vegetarian smokers, though the differences were mostly insignificant. There were, however, highly significant differences ( $P < 0.01$ ) between vegetarian and non-vegetarian non-smokers and between vegetarian and non-vegetarian smokers. It was obvious that the nature of the diet was responsible for the differences between the four main groups and not the smoking habit. This was clearly confirmed when the mean value of A in smokers, regardless of diet, was not found to be significantly different from that in non-smokers, whereas the level in vegetarians, regardless of smoking habit, was found to be highly significantly lower than that in non-vegetarians (Table II).

The mean value of A for all subjects taken together was 292 pg/ml (range 105–728 pg/ml) (Table II). When the patients' relatives were considered separately from the laboratory workers and doctors, however, the former showed a significantly lower mean value than the latter (Table II, third column); the ranges were 105–365 pg/ml and 154–728 pg/ml respectively.

There was no significant difference between smokers and non-smokers or between vegetarians and non-vegetarians in respect of non-CN-extracted serum B<sub>12</sub> (value B). There were, however, significant differences both between smokers and non-smokers and between vegetarians and non-vegetarians when B was expressed as a percentage of A ( $B/A \times 100$ ) (Table II). Vegetarianism as well as smoking appeared to bring about a rise of  $B/A \times 100$ . This is discussed below.

TABLE I—Serum Vitamin B<sub>12</sub>, Thiocyanate, and Folate Levels in Four Groups of Normal Subjects (Mean  $\pm$  S.D.). Numbers of Subjects are given in Parentheses

Groups	Serum Total (CN-extracted) B <sub>12</sub> (pg/ml) (A)	Serum Non-CN-extracted B <sub>12</sub> (pg/ml) (B)	B/A $\times$ 100	Plasma Thiocyanate ( $\mu$ mol/100 ml)	Serum Folate (ng/ml)	
Non-vegetarian non-smokers	365 $\pm$ 200 (26) $\uparrow$	$\left\{ \begin{array}{l} 415 (15) \\ 297 (11) \\ 365 (10) \\ 263 (9) \\ 213 (9) \\ 190 (6) \\ 210 (5) \\ 127 (4) \end{array} \right\}$	141 $\pm$ 80 (25)	39 $\pm$ 5.7 $\uparrow$	1.75 $\pm$ 0.83 (25) $\S$	5.01 $\pm$ 2.27 (20) $\uparrow$
Non-vegetarian smokers	314 $\pm$ 161 (19) $\ddagger$		150 $\pm$ 60 (14)	48 $\pm$ 10.1 $\uparrow$	8.86 $\pm$ 4.43 (15) $\S$	4.50 $\pm$ 2.18 (13) $\uparrow$
Vegetarian non-smokers	204 $\pm$ 45 (15) $\uparrow$		103 $\pm$ 38 (14)	51 $\pm$ 14.3 $\uparrow$	2.47 $\pm$ 0.77 (15) $\S$	6.45 $\pm$ 4.02 (12) $\uparrow$
Vegetarian smokers	173 $\pm$ 68 (9) $\uparrow$		104 $\pm$ 39 (8)	60 $\pm$ 5.3 $\uparrow$	6.98 $\pm$ 3.24 (8) $\S$	6.85 $\pm$ 1.37 (7) $\uparrow$

\*  $P < 0.01$ . $\uparrow$  N.S. $\ddagger$   $P < 0.05$ . $\S$   $P < 0.001$ .

Paired figures in column 3 indicate the two different types of subject (see text).

TABLE II—Comparison between Smokers and Non-smokers and between Vegetarians and Non-vegetarians (Mean  $\pm$  S.D.). Numbers of Subjects are given in Parentheses

Groups	Serum Total (CN-extracted) B <sub>12</sub> (pg/ml) (A)	Serum Non-CN-extracted B <sub>12</sub> (pg/ml) (B)	B/A $\times$ 100	Plasma Thiocyanate ( $\mu$ mol/100 ml)	Serum Folate (ng/ml)	
Non-smokers	306 $\pm$ 179 (41) $\uparrow$	$\left\{ \begin{array}{l} 339 (24) \\ 259 (17) \\ 313 (15) \\ 221 (13) \\ 395 (25) \\ 280 (20) \\ 212 (14) \\ 165 (10) \end{array} \right\}$	128 $\pm$ 70 (39)	42 $\pm$ 10.7 $\ddagger$	2.02 $\pm$ 0.95 (40)	5.29 $\pm$ 3.07 (32)
Smokers	270 $\pm$ 152 (28) $\uparrow$		133 $\pm$ 58 (22)	49 $\pm$ 10.9 $\uparrow$	8.20 $\pm$ 4.10 (23)	5.55 $\pm$ 2.23 (20)
Non-vegetarians	345 $\pm$ 185 (45) $\uparrow$	$\left\{ \begin{array}{l} 329 \pm 187 (39) \\ 243 \pm 129 (30) \end{array} \right\}$	145 $\pm$ 73 (39)	42 $\pm$ 7.9 $\uparrow$	4.41 $\pm$ 4.40 (40)	4.79 $\pm$ 2.21 (33) $\ddagger$
Vegetarians	192 $\pm$ 57 (24) $\uparrow$		103 $\pm$ 38 (22)	54 $\pm$ 12.9 $\uparrow$	3.00 $\pm$ 2.93 (23)	6.60 $\pm$ 3.24 (19) $\ddagger$
All subjects	292 $\pm$ 169 (69)	130 $\pm$ 65 (61)	45 $\pm$ 11.5	4.28 $\pm$ 3.93 (63)	5.45 $\pm$ 2.74 (52)	

\* N.S.

 $\uparrow$   $P < 0.01$ . $\ddagger$   $P < 0.05$ .

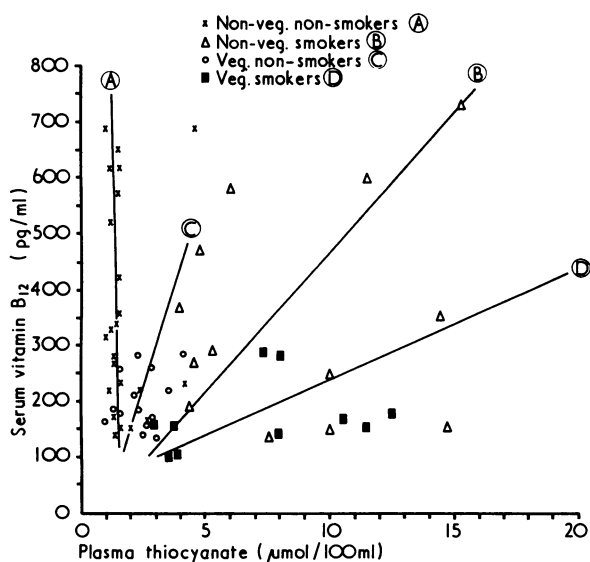
Paired figures in column 3 indicate the two different types of subject (see text).

For all subjects  $B/A \times 100$  ranged from 24 to 72%, with a mean of 45%. This compares with the range of 28-78% with a mean of 51% reported by Matthews (1961) using *L. leichmannii* assay. The highest mean value of  $B/A \times 100$  was among vegetarian smokers (60%) and the lowest among non-vegetarian non-smokers (39%) (Table I). Moreover, the two vegetarian smokers with the lowest total  $B_{12}$  values (105 pg/ml each) also showed raised values of  $B/A \times 100$  (60 and 62%, respectively). On the other hand, in a non-vegetarian smoker with a very high plasma thiocyanate level (see below) B was only 37% of A.

#### PLASMA THIOCYANATE

As expected, plasma thiocyanate values were highly significantly raised in smokers when compared with non-smokers (Table II), and in vegetarian and non-vegetarian smokers separately (Table I). There was no significant difference between vegetarians and non-vegetarians (Table II). The lowest individual value was 1.0  $\mu\text{mol}/100\text{ ml}$  and the highest 15.4  $\mu\text{mol}/100\text{ ml}$  (in a young man of 20 who had been smoking "charas," a preparation of *Cannabis indica*, in his cigarettes).

The actual serum total  $B_{12}$  and plasma thiocyanate values in



Correlation between plasma thiocyanate and serum total vitamin  $B_{12}$

all the 69 subjects are shown in the Chart. It is seen that non-vegetarian non-smokers, vegetarian non-smokers, non-vegetarian smokers, and vegetarian smokers, form interdigitating groups, with a trend in that order towards a fall in serum  $B_{12}$ , and a rise in plasma thiocyanate levels.

#### SERUM FOLATE

Smokers and non-smokers did not differ significantly in respect of their serum folate levels, but the mean level was slightly but significantly higher in vegetarians than in non-vegetarians (Table II). The lowest individual value was 2.75 ng/ml and the highest 15.75 ng/ml; both subjects happened to be vegetarians.

#### Discussion

This appears to be the first time that vitamin  $B_{12}$ , thiocyanate, and folate levels in the blood have been estimated in separate groups of vegetarian and non-vegetarian smokers and non-smokers. Serum  $B_{12}$  levels in the normal population have been

estimated by microbiological assay by several groups of workers from India and the West (Mollin and Ross, 1952; Dixit *et al.*, 1956; Herbert *et al.*, 1960; Satoskar *et al.*, 1961; Gupta, 1965; Baker and Frank, 1968; Jathar *et al.*, 1970). However, only Satoskar *et al.*, Gupta, and Jathar *et al.* gave comparative values for vegetarians and non-vegetarians, the former being the lower, and they did not study the relation of  $B_{12}$  levels to smoking.

The significantly lower serum total  $B_{12}$  values found by us in vegetarians than in non-vegetarians are probably a reflection of the lower intake of the vitamin on their part, meat products being rich in  $B_{12}$ . Vegetarians who can eat adequate amounts of milk and nuts, which are also rich in  $B_{12}$ , can maintain adequate serum levels. People from the lower socioeconomic groups, such as those of the apparently normal subjects who were drawn from among the relatives of the generally poor patients in our free hospital, would be expected to have a deficient intake of all these relatively expensive articles of diet and therefore to have lower serum  $B_{12}$  levels. This is precisely what was seen in this group, among whom non-vegetarians as well as vegetarians had significantly lower  $B_{12}$  values than their counterparts among the more prosperous laboratory workers and doctors (Table II).

In 11 vegans Smith (1962) found serum  $B_{12}$  values ranging from 48 to 190 pg/ml and suggested that they had remained free of symptoms of any central nervous system disorder because the major portion of the  $B_{12}$  in their serum was in the hydroxo form. He further made a comparison between veganism and tobacco amblyopia and invoked endogenous and exogenous factors interfering with cyanide metabolism in both these conditions. In a vegan who did not receive any  $B_{12}$  supplement Linnell *et al.* (1969) found a serum  $B_{12}$  value of 100 pg/ml, measured by the isotopic assay that we also used. This is comparable to the value of 105 pg/ml in two of our vegetarian smokers.

Smith (1961a, 1961b) stated that in patients with tobacco amblyopia and in heavy pipe smokers there was no significant difference between the serum  $B_{12}$  values obtained with and without added cyanide, whereas the difference was highly significant in non-smokers. He suggested that this difference represents the amount of hydroxocobalamin in the blood. The effect of smoking on the serum  $B_{12}$  level is a phenomenon not amenable to any facile evaluation. Thus in our subjects the small and statistically insignificant reduction in value A in the smokers as compared with the non-smokers has to be weighed against the virtual absence of any difference in value B between these two subgroups (Table II). Hence it would appear that the statistically significant rise of  $B/A \times 100$  among smokers is a reflection of the reduction in value A as compared with non-smokers. Wilson and Matthews (1966) reported similar findings.

It is of interest that we also found the value of B in relation to A to be significantly higher in vegetarians than in non-vegetarians irrespective of smoking habit. Hence smoking was not the sole factor producing a rise of  $B/A \times 100$ . To find an explanation of this the possible nature of values A and B must be discussed.

The conversion of the various forms of vitamin  $B_{12}$  to that form used as the standard—cyanocobalamin—is an essential step in the isotopic assay in order to avoid any differential binding of the true forms of  $B_{12}$  to the binding agent. This may also hold good for microbiological assays if the responses of the microbe to the various forms of the vitamin differ. CN-extracted  $B_{12}$  (value A) and non-CN-extracted  $B_{12}$  (value B), as determined by us or by Wilson and Matthews (1966), obviously do not represent any true forms of vitamin  $B_{12}$  in the blood. As shown originally by Lindstrand and Stahlberg (1963) and Stahlberg (1964) in normal subjects and by Linnell *et al.* (1969) in both normal subjects and patients with pernicious anaemia, unidirectional chromatography coupled with bioautography shows two constantly present forms of  $B_{12}$ —namely, methylcobalamin and a combination of hydroxocobalamin with deoxyadenosylcobalamin. Recently the latter two have been separated by two-directional chromatography (Linnell *et al.*, 1970).

Cyanocobalamin certainly does not represent the total

cobalamins and may be present in very small amounts or virtually absent. Lindstrand *et al.* (1966) could detect cyanocobalamin in only a minority of smokers and, when detected, it was present in relatively small amounts. Hence the conclusion is inescapable that the total (CN-extracted) B<sub>12</sub> in the blood is not cyanocobalamin, as believed at one time (Wokes and Picard, 1955), but perhaps a combination of all the true forms of B<sub>12</sub>, mentioned above. The concentration of true cyanocobalamin in the blood may be raised in tobacco amblyopia and hereditary optic neuropathies in the presence of a normal total B<sub>12</sub> level; this rise is thought to be functionally unimportant (Wilson *et al.*, 1971; Matthews 1971). The other important feature shown by bioautography is that methylcobalamin is biologically the most important fraction of vitamin B<sub>12</sub>, and that this is the fraction which is depleted in pernicious anaemia and not hydroxocobalamin, which may in some cases be the predominant component (Linnell *et al.*, 1969).

In view of this, the earlier suggestion of Smith (1961a, 1961b) that the difference between values A and B represents the amount of hydroxocobalamin in the blood and that this is the physiologically active fraction concerned with the detoxication of cyanide appears untenable. What values A and B actually represent in terms of the different forms of cobalamins now discovered is still unclear. It seems plausible that value A, as obtained in the present investigation, represents all the protein-bound B<sub>12</sub>, together with any present in the free form. It is now known that there are at least two B<sub>12</sub>-binding proteins in the blood, transcobalamin I, an  $\alpha$ -globulin described by Pitney *et al.* (1954), and transcobalamin II, a  $\beta$ -globulin described by Hall and Finkler (1963). Hall and Finkler (1965) later suggested that transcobalamin I might be concerned with the transport of pre-existing endogenous B<sub>12</sub>, while the main function of transcobalamin II might be the transport of B<sub>12</sub> shortly after its intake into the body. It is also not clear what value B represents in terms of B<sub>12</sub>, bound to transcobalamin I and transcobalamin II or to any other plasma protein. It appears possible that value B, as assayed with *L. leichmannii* or radioisotopically, represents free B<sub>12</sub>.

Our finding that smoking is certainly not the sole factor, and perhaps not the most important factor, in producing a rise of B in relation to A suggests the possibility that B and B/A  $\times$  100 may not be mere reflections of increased levels of cyanide or thiocyanate in the blood. The fact that we found B/A  $\times$  100 to be maintained at a high level of about 60% even when the total value (A) had fallen as low as 105 pg/ml (in two vegetarian smokers) could suggest that B represents a physiologically active moiety which the body tries to maintain until a critical level of the total value is reached. This suggestion gains support from our recent (unpublished) finding in three patients with B<sub>12</sub> neuromyelopathy of abnormally low values of A as well as of B/A  $\times$  100 (<20%). In two of them, on whom successive

estimations could be carried out in the course of B<sub>12</sub> therapy, there was first a gradual rise of B/A  $\times$  100 to abnormally high levels (90%), followed a week after the last injection by a return to the normal level (50%).

Nothing need be said about the serum folate levels observed except that the significantly higher value in vegetarians than in non-vegetarians (Table II) is probably a reflection of the higher intake of folates in a vegetarian diet.

We are most grateful for grant No. 01-011-1 from the National Institutes of Health of the U.S. Department of Health, Education, and Welfare for supporting a research project on nutritional disorders of the nervous system, as part of which this work was carried out.

## References

- Aldridge, W. N. (1945). *Analyst*, 70, 474.  
 Baker, H., and Frank, O. (1968). *Clinical Vitaminology*. New York, Interscience.  
 Boger, W. D., Wright, L. D., Strickland, S. C., Gybe, J. S., and Ciminera, J. I. (1955). *Proceedings of the Society for Experimental Biology and Medicine*, 89, 375.  
 Boxer, G. E., and Rickards, J. C. (1952). *Archives of Biochemistry*, 39, 7.  
 Dixit, C. H., Mody, B. M., Jhala, H. I., Parekh, J. G., and Ramasarma, G. B. (1956). *Indian Journal of Medical Sciences*, 10, 419.  
 Gupta, O. P. (1965). *Journal of the Indian Medical Association*, 45, 362.  
 Hall, C. A., and Finkler, A. E. (1963). *Biochimica et Biophysica Acta*, 78, 233.  
 Hall, C. A., and Finkler, A. E. (1965). *Journal of Laboratory and Clinical Medicine*, 65, 459.  
 Heaton, J. M., McCormick, A. J. A., and Freeman, A. G. (1958). *Lancet*, 2, 286.  
 Herbert, V., *et al.* (1960). *Blood*, 51, 228.  
 Jathar, V. S., Patrawalla, S. P., Doongaji, D. R., Rege, D. V., and Satoskar, R. S. (1970). *British Journal of Psychiatry*, 117, 699.  
 Lindstrand, K., and Stahlberg, K. G. (1963). *Acta Medica Scandinavica*, 174, 665.  
 Lindstrand, K., Wilson, J., and Matthews, D. M. (1966). *British Medical Journal*, 2, 988.  
 Linnell, J. C., Hussein, H. A. A., and Matthews, D. M. (1970). *Journal of Clinical Pathology*, 23, 820.  
 Linnell, J. C., Mackenzie, H. M., Wilson, J., and Matthews, D. M. (1969). *Journal of Clinical Pathology*, 22, 545.  
 Matthews, D. M. (1961). *Lancet*, 1, 1289.  
 Matthews, D. M. (1962). *Clinical Science*, 22, 101.  
 Matthews, D. M. (1971). *British Medical Journal*, 3, 659.  
 Matthews, D. M., Gunasegaram, R., and Linnell, J. C. (1967). *Journal of Clinical Pathology*, 20, 683.  
 Mollin, D. L., and Ross, G. I. M. (1952). *Journal of Clinical Pathology*, 5, 129.  
 Pitney, W. R., Beard, M. F., and Van Loon, E. J. (1954). *Journal of Biological Chemistry*, 207, 143.  
 Satoskar, R. S., Kulkarni, B. S., and Rege, D. V. (1961). *Indian Journal of Medical Research*, 49, 887.  
 Smith, A. D. M. (1961a). *Lancet*, 1, 1001.  
 Smith, A. D. M. (1961b). *Lancet*, 1, 1346.  
 Smith, A. D. M. (1962). *British Medical Journal*, 1, 1655.  
 Spray, G. H. (1955). *Clinical Science*, 14, 661.  
 Stahlberg, K. G. (1964). *Scandinavian Journal of Haematology*, 1, 220.  
 Wilson, J., Linnell, J. C., and Matthews, D. M. (1971). *Lancet*, 1, 259.  
 Wilson, J., and Matthews, D. M. (1966). *Clinical Science*, 31, 1.  
 Wokes, F. (1958). *Lancet*, 2, 526.  
 Wokes, F., and Picard, C. W. (1955). *American Journal of Clinical Nutrition*, 3, 383.